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THE ASSOCIATION BETWEEN OXIDATIVE STRESS AND ONE CARBON METABOLISM IN PROSTATE NEOPLASM IN A TERTIARY CARE HOSPITAL IN NORTH INDIA

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Thesis submitted to

SANTOSH DEEMED TO BE UNIVERSITY

(Established u/s 3 of the UGC Act, 1956)

For the Award of degree of DOCTOR OF MEDICINE IN BIOCHEMISTRY

By

DR. SWETA KUMARI

(2020-2021)

Under the guidance of

DR. JUHI AGGARWAL, PROFESSOR AND HEAD

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SANTOSH MEDICAL COLLEGE & HOSPITAL,

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Dated, Ghaziabad …… 2022 ***(DR. SWETA KUMARI)***

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CHAPTER: 1

INTRODUCTION

1 INTRODUCTION

Carcinoma of the prostate is the most common malignant tumor in men over the age of 65 years with peak age of incidence between 70 and 74 years.[1] In 2017, ~161,360 prostate cancer cases were diagnosed and 26,730 men died from prostate cancer in the United States. The disease is more common in countries with higher proportions of elderly men in their population. Incidence of prostate cancer in India is lower than that in the Western populations. Most early-stage prostate cancers are asymptomatic and difficult to detect clinically. The disease either becomes locally advanced or metastatic. The absolute number of prostate cancer deaths has decreased in the past 10 years, attributed by some to the widespread use of PSA-based detection strategies. However, the paradox of management is that although 1 in 6 men will eventually be diagnosed with prostate cancer, and the disease remains the second leading cause of cancer deaths in men, only 1 man in 30 with prostate cancer will die of his disease.[2] The progressive inherent or acquired changes in cellular metabolism occurring with aging may play an important role in the development of this disease.[3]

In Prostate Cancer, one-carbon metabolism pathway is modulated by the Androgen Receptor. This pathway is comprised of

a number of connected pathways that promote the folate-mediated transfer of one-carbon units which are necessary for essential cellular processes, including DNA synthesis, repair and the maintenance of redox status.[4,5,6]

Etiopathogenesis of prostate cancer includes oxidative stress and one carbon metabolism. The prooxidant-antioxidant balance of prostate cancer is shifted towards a state of oxidative stress when compared to normal prostate.[7]

"Oxidative stress" is the state of a cell, characterized by excess production of reactive oxygen species (ROS) and/or a reduction in antioxidant defenses mechanism responsible for metabolism. ROS are formed as a natural by-product of the normal oxygen metabolism. Under normal circumstances, a cell is able to maintain an adequate homeostasis between the formation of ROS and its removal through enzymatic pathways or via antioxidants.[8] If this balance is disturbed, oxidative stress occurs. This generates an imbalance of production/removal of ROS, which is either directly or indirectly involved in initiation, promotion, and progression phases of carcinogenesis.[9] Oxygen radicals may cause damage to DNA and chromosomes, induce epigenetic alterations, interact with oncogenes or tumor suppressor genes, and impart changes in immunological mechanisms.[10,11]

Folate is necessary for DNA synthesis, repair and methylation, and biological reactions involving folate require vitamins B_6 and B_{12} as cofactors. Low concentrations of these vitamins can impair one-carbon metabolism pathways,[12] leading to homocysteine accumulation, insufficient methyl groups for DNA methylation or depletion of DNA synthesis and repair precursors, thus, potentially promoting carcinogenesis.[13] These nutrients have been implicated with respect to several malignancies, including colorectal, lung, cervix[14] and may impact prostate cancer as well.[15] Both hyper- and hypomethylated DNA have been found in prostate tumors,[16] and the activation of oestrogen receptors in prostate cancer cell lines and tissues is regulated through promoter region methylation.[16]

Folate in the form of 5,10-methyltetrahydrofolate may have a protective role in cancer development by promoting the synthesis of thymidylate from uracil, minimizing the misincorporation of uracil into DNA. Excessive uracil could lead to double-strand breaks and possibly to cancer development.[17]

Level of MDA (Malondialdehyde) was increased in prostate cancer patients, compared to healthy controls[18] and patients with benign prostate hyperplasia.[19] It is proposed that the decreased levels of MDA, may relate to tumor aggressiveness, in a way that

rapidly dividing cells tend to set an oxidant antioxidant status favorable to their growth.[20]

Elevated homocysteine may promote oxidative stress by inhibiting the expression and activity of glutathione peroxidases. Elevated plasma homocysteine levels, a condition that may also be associated with folate deficiency, is often seen in the setting of malignancy. In addition to being a metabolite that is utilized in glutathione synthesis, homocysteine regulates the activity of enzymes that use glutathione as a cofactor. By controlling glutathione synthesis and utilization, changes in one-carbon metabolism flux can have a profound impact on redox metabolism, and therefore, potentially tumorigenesis and cancer progression.[21]

Keeping above background, parameters like MDA (for oxidative stress), Vitamin B_{12} and homocysteine (for status of the one carbon metabolism) were selected for the present study to investigate some of the multifactorial etiology of prostate neoplasms. Study on these parameters in prostate neoplasms are very few in number and the results are also far from any definite conclusion. Hence, present study aims to compare serum level of these parameters between control, and prostate neoplasm cases to find any association.

[redacted]***[redacted]

CHAPTER: 2

AIMS & OBJECTIVES

2 AIMS AND OBJECTIVES

AIM:

❖ To compare serum level of Oxidative Stress and One Carbon Metabolism between control and prostate neoplasm cases to find any association.

OBJECTIVES:

➢ To study Serum level of MDA in Prostate Neoplasm Patients.

➢ To study Serum level of Vitamin B12 in Prostate Neoplasm Patients.

➢ To study Serum level of Homocysteine in Prostate Neoplasm Patients.

➢ To compare level of Oxidative Stress Marker and One Carbon Metabolism markers between Prostate Neoplasm Patients and Control Group.

RESEARCH QUESTION:

The purpose of the intended study is to find out whether Oxidative Stress and One Carbon Metabolism are important contributory factors in Prostate Neoplasm.

HYPOTHESIS:

To study the association of Oxidative Stress and One Carbon Metabolism as important contributory factors in Prostate Neoplasm is helpful in monitoring the risk of Prostate Neoplasm in initial stage.

NULL HYPOTHESIS:

To study the association of Oxidative Stress and One Carbon Metabolism as important contributory factors in Prostate Neoplasm is not helpful in monitoring the risk of Prostate Neoplasm in initial stage.

SCOPE OF THE STUDY:

This study is intended to see the association between Oxidative Stress and One Carbon Metabolism in Prostate Cancer as contributing factors.

Parameters like MDA (for oxidative stress), Vitamin B12 and homocysteine (for status of the one carbon metabolism) were selected for the present study to investigate some of the multifactorial etiology of prostate neoplasms. Study on these parameters in prostate neoplasms are very few in number and the results are also far from any definite conclusion. Hence, present study aims to compare serum level of these parameters between control, BPH and prostate cancer cases to find any association.

The study suggests that high level of oxidative stress and One carbon Metabolism is associated with prostate cancer cases compared to age matched control group. Possible role of antioxidant medications in modulating outcome in these patients, however needs exploration by randomized trials.

It was found that there was an association of high Homocysteine level and tumors of prostate origin whereas with vitamin B12, no association was documented.

Association of hyper Homocysteinemia with prostate cancer, if proved by larger prospective studies, may indicate the emergence of Hcy as a novel tumor marker.

The study basically focuses on all the above points mentioned so that the results observed may be helpful in diagnosing prostate cancer in early stage.

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CHAPTER: ३

REVIEW OF LITERATURE

3 REVIEW OF LITERATURES

PROSTATE

2.1.1 Prostate Anatomy:

The prostate is a gland of the male reproductive system. In adults, it is about the size of a walnut,[22] and has an average weight of about 11 grams, usually ranging between 7 and 16 grams.[23] The prostate is located in the pelvis. It sits below the urinary bladder and surrounds the urethra. The part of the urethra passing through it is called the prostatic urethra, which joins with the two ejaculatory ducts.[22] The prostate is covered in a surface called the prostatic capsule or prostatic fascia.[24]

The internal structure of the prostate has been described using both lobes and zones.[22,25] Because of the variation in descriptions and definitions of lobes, the zone classification is used more predominantly.[22]

The prostate has been described as consisting of three or four zones.[22,26] Zones are more typically able to be seen on histology, or in medical imaging, such as ultrasound or MRI.[22,25] The zones are:

Zone	Details
Peripheral zone	This is the back of the gland that surrounds the distal urethra and lies beneath the capsule.
Central zone	This is the zone that surrounds the ejaculatory duct.
Transition zone	This zone surrounds mainly proximal urethra
Anterior fibro-muscular zone	This zone is composed of muscular and fibrous tissue and doesn't have glandular component

Table 3.1: Zones of Prostate [22,26]

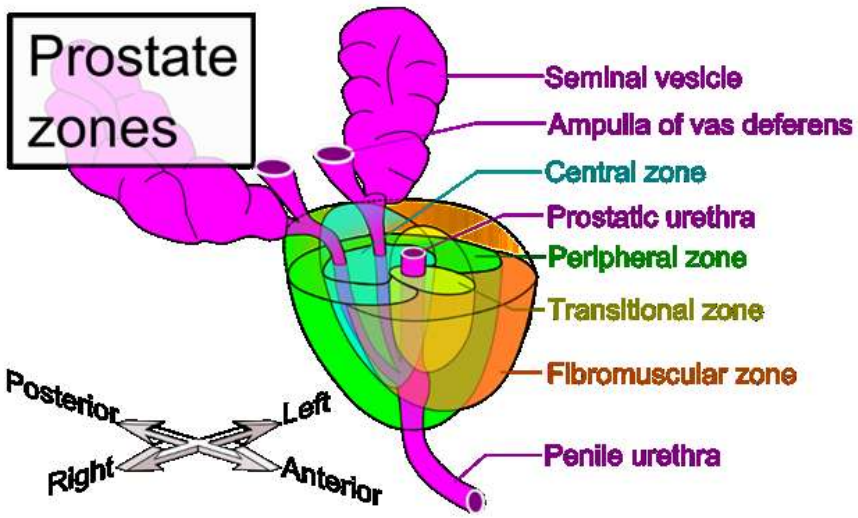

Image 2.1: Zones of Prostate

2.1.2 Microanatomy of Prostate:

Prostate consists of glandular tissue as well as connective tissue.[22] The epithelium is mainly composed of column shaped cells which may be in a single layer or pseudo-stratified.[22] Cuboidal and flat cells are also present along with transitional epithelium lining the ducts. The glands are made of follicles, draining into canals and finally into 10/12 large ducts. These in turn drain into urethra.[26]

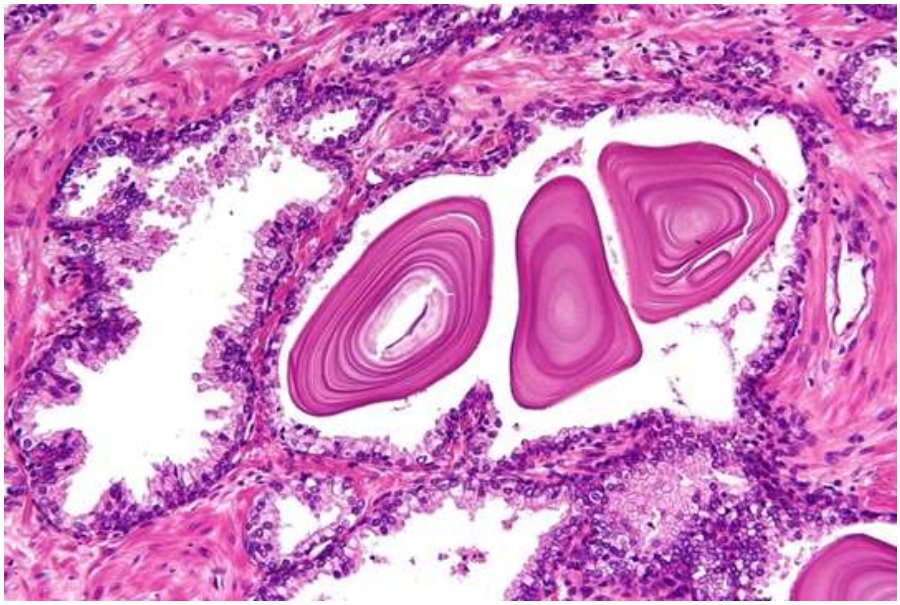

Image 2.2: Zones of Prostate

2.1.3 Blood Supply and Lymphatic Drainage:

The blood supply of prostate is through inferior vesical artery, internal pudendal artery, and middle rectal arteries. These vessels enter the prostate on its outer surface where it, and travel forward to the apex of the prostate.[26] Both the inferior vesical and the middle rectal arteries arise together directly from the internal iliac arteries. After entering the bladder, the inferior vesical artery splits into a urethral branch and a capsular branch, former supplies the urethral prostate; and the later travels around the capsule with smaller branches which perforate into the prostate.[26]

The veins of the prostate form the prostatic venous plexus, primarily around its front and outer surface.[26] This network receives blood from the deep dorsal vein of the penis and is connected to the vesical plexus and internal pudendal veins. Veins drain into the vesical and then internal iliac veins.[26]

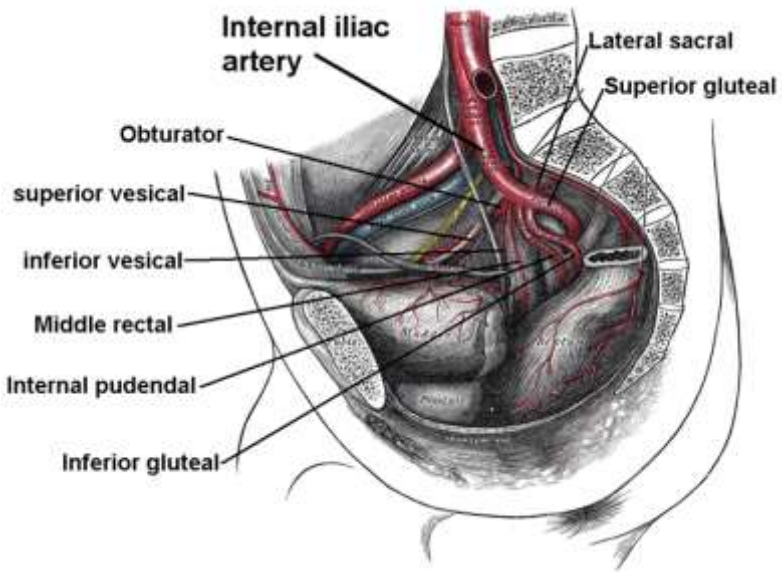

Image 2.3: Blood supply of Prostate

The lymphatic vessels surrounding the vas deferens, some of the vessels in the seminal vesicle, and a vessel from the posterior surface of the prostate drain into the external iliac lymph nodes.[26] Some of the seminal vesicle vessels, prostatic vessels, and vessels from the anterior prostate drain into internal iliac lymph nodes. Vessels of the prostate itself also drain into the obturator and sacral lymph nodes.[26]

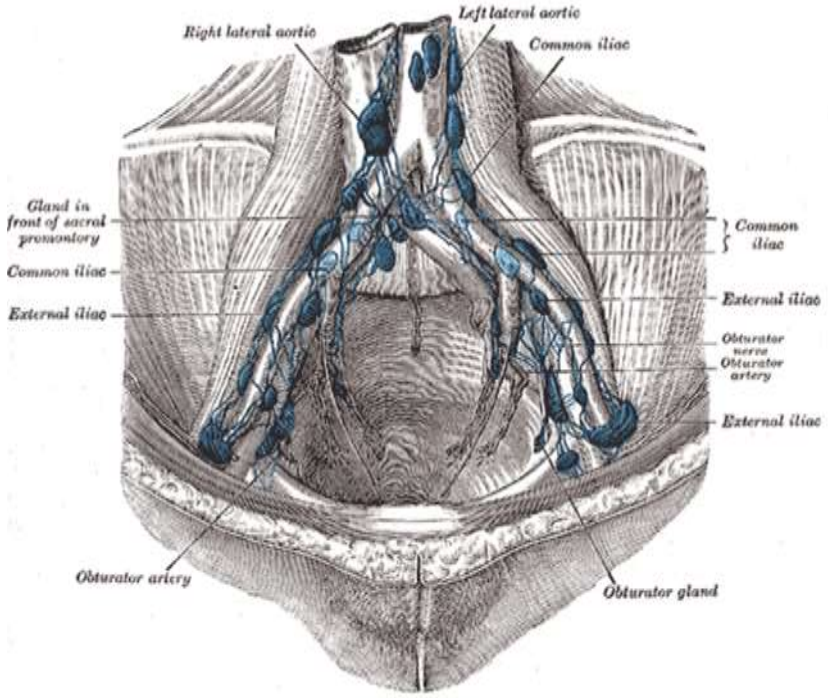

Image 2.4: Lymphatic drainage of Prostate

2.1.4 Function of Prostate Gland:[26,27]

The prostate secretes fluid which form a part of semen. Semen is the fluid ejaculated by males during the sexual response. Ejaculation is the expulsion of semen from the urethra. When semen is ejaculated, it is transmitted from the vas deferens into the male urethra via the ejaculatory ducts. This occurs following contractions of the smooth muscle of the vas deferens and seminal vesicles, due to stimulation, primarily of the glans penis. Stimulation sends nerve signals via the internal pudendal nerves to the upper lumbar spine. After traveling into the urethra, the seminal fluid is ejaculated by contraction of the bulbocavernosus muscle. The secretions of the prostate contain proteolytic enzymes, prostatic acid phosphatase, fibrinolysin, zinc, and prostate-specific antigen.

The prostate's changes of shape, helping the mechanical switch between urination and ejaculation. These are mainly driven by the two longitudinal muscle systems running along the prostatic urethra.

2.2.1 Prostate Neoplasm

Carcinoma of the prostate is the most common malignant tumour in men over the age of 65 years with peak age of incidence between 70 and 74 years.[1] Most prostate cancers are slow growing. Cancerous cells may metastasise to other areas of the body, particularly by hematogenous spread to the bones or via lymphatic spread.

2.2.2 Sign & Symptoms of Prostate Cancer:[1,2]

Prostate cancer may initially cause no symptoms. In later stages, symptoms include

- Dull pain in the lower pelvic area
- Frequent urinating
- Trouble urinating, pain, burning, or weak urine flow
- Blood in the urine (Haematuria)
- Painful ejaculation
- Pain in the lower back, hips or upper thighs
- Loss of appetite

- Loss of weight

2.2.3 High Risk factors for Prostate Cancer:[28,29]

Age

As age advances, their risk of getting prostate cancer increases. It is rarely found in men younger than age 40. Damage to the genetic material (DNA) of prostate cells is more likely for men over the age of 55. Damaged or abnormal prostate cells can begin to grow out of control and form tumours.

Ethnicity

African American men have, by far, the highest incidence of the disease. One in six African American men will get prostate cancer. They are also more like to have aggressive tumours that grow quickly, spread and cause death. Other ethnicities, such as Hispanic and Asian men, are less likely to get prostate cancer.

Family History

Men with a family history of prostate cancer also face a higher risk of also developing the disease. First degree relatives of prostate cancer patients have two to three times higher risk. This risk increases with the number of relatives diagnosed with the disease. The age at which a close relative was diagnosed is also an important factor.

Smoking

Studies show prostate cancer risk may double for heavy smokers. Smoking is also linked to a higher risk of dying from prostate cancer.

Diet

Diet and lifestyle may affect the risk of prostate cancer. A diet rich in calories, animal fats, refined sugar but not enough fruits and vegetables imparts high risk of developing prostate cancer. A lack of exercise is also linked to poor outcomes. Obesity is known to increase a man's risk of dying from prostate cancer.

2.2.4 Pathophysiology of Prostate Cancer:

The prostate is part of the male reproductive system that helps make and store seminal fluid. Superiorly, the prostate base is contiguous with the bladder outlet. Inferiorly, the prostate's apex is towards the direction of the urogenital diaphragm, pointed anterio-inferiorly.[30] The prostate can be divided into four anatomic spaces: peripheral, central and transitional zones, and anterior fibromuscular stroma.[22,26]

Most of the glandular tissue is found in the peripheral and central zones. Around 70-80% of glandular tissue is present in peripheral zone and

around 20% in central zone.[31] Approximately 5% of glandular tissue is also found in the transitional zone Thus, most cancers originating from glandular tissue are found in the peripheral and central spaces while about 5% is found in the transitional space. None cancer is found in the anterior fibromuscular stroma since no glands are in that anatomic space.[32]

Cancers originating from glandular tissue (called as adenocarcinomas), which develop by mutation of semen-secreting glandular cells are the most common prostate cancer.[33] Initially, small clumps of cancer cells remain within otherwise normal prostate glands, a condition known as carcinoma in situ or prostatic intraepithelial neoplasia (PIN).[34] Prostatic intraepithelial neoplasia is closely associated with cancer.[35] Eventually tumours grow in size invading nearby structures like seminal vesicle and rectum. They may also spread to distant organs through metastasis by blood or lymphatic spread.[36]

Prostate is an organ that accumulates zinc and produces citrate. This action of zinc accumulation and citrate production require enormous amount of energy in the form of ATP. Prostate cancer cells are generally devoid of zinc and they save energy by avoiding citrate production. This energy is eventually used by the tumour cells to grow and metastasise.[37]

Cancer suppressor genes, supposed to be lost early in prostatic carcinogenesis, have been identified as chromosomes 8p, 10q, 13q, and 16q. p53 mutations are relatively low in the primary prostate cancer and are more frequently seen in metastatic carcinoma only. Other tumour suppressor genes that are thought to be involved PTEN and KAI1. It is believed that “Up to 70 percent

of men with prostate cancer have lost one copy of the PTEN gene at the time of diagnosis".[38] Relative frequency of loss of E-cadherin and CD44 have also been observed. Loss of the retinoblastoma protein induces so-called androgen receptor deregulation in prostate cancer mainly by deregulating 'E2F1 expression.[39]

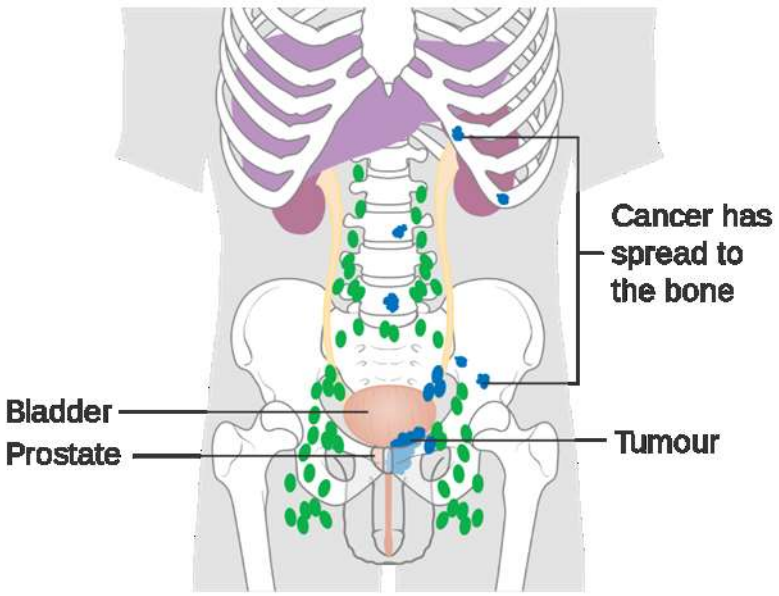

Image 2.5: Metastasis of Prostate cancer

OXIDATIVE STRESS AND PROSTATE CANCER

2.3.1 Historical Review

Klotz et al (1988) in their study on patients of prostate cancer and benign prostatic hyperplasia observed high iNOS expression in prostate carcinoma tissues compared to BPH and suggested that epithelial iNOS expression can be used as a specific immunohistochemical marker in prostate cancer patients.[40]

Baltaci et al (2004) in their study on Prostatic Intraepithelial Neoplasia (PIN) and prostate cancer patients observed positive iNOS immunostaining in all samples from all patients. They also observed more intense expression in prostate carcinoma and high-grade PIN patients as compared to low grade PIN patients and BPH patients.[41]

Camphausen et al (2004) in their study on Prostate cancer patients, did not observe any increase in either 8-iso-PGF 2 or 15-keto-dihydro PGF2 in urine of patients with cancer as compared to normal controls.[42]

Iynem et al (2004) conducted a study on metastatic prostate cancer patients observed significantly high plasma MDA levels and low vitamin E levels in patients compared to controls.[43]

Yilmaz et al (2004) in their study on newly diagnosed prostate cancer and treated BPH patients observed high MDA levels and low zinc levels as well as

low antioxidant activity in cancer patients as compared to BPH patients and healthy controls.[44]

Miyake et al (2004) conducted their study on prostate cancer (stage T1 – T4) patients and measured urinary 8-OHdG and creatinine levels as a marker of oxidative damage. They observed urinary 8-OHdG to creatinine ratio was significantly higher in patients compared to controls. They also observed that there was no significant difference in urinary 8-OHdG to creatinine ratio before and after radical prostatectomy. However, ration was significantly lower after hormonal therapy compared to before therapy.[45]

Srivastava et al in 2005 observed significantly higher levels of MDA and GSTs activity in serum of prostate cancer and BPH patients when compared to healthy controls.[46]

Almushatat e al (2006) found that prostate cancer patients had higher levels of MDA and lower levels of circulating lutein, lycopene and β-carotene. α-tocopherol, retinol, lutein and lycopene were observed in metastatic disease and compared to localized tumor.[47]

Aydin et al in their study (2006) on newly diagnosed patients of prostate cancer and BPH patients observed increased lipid peroxidation in patients versus controls and cancer patient versus patients with BPH.[48]

Ozmen et al (2006) found that vitamin A, C and E levels were significantly lower and MDA levels were higher in patients that controls. They

also observed that selenium and zinc levels were lower whereas nickel, copper and cobalt levels were higher in patients compared to controls.[49]

Surapanenei et al (2006) found erythrocyte MDA and SOD levels to be significantly higher in prostate cancer patients than in controls.[50]

Yossepowitch et al (2007) suggested that oxidative stress indices had no difference compared to healthy controls in patients with localized tumor whereas those with metastatic diseases had significantly higher levels of MDA.[51]

Kotrikadze et al in 2008 demonstrated that plasma MDA levels were significantly elevated both in BPH patients and in patients with carcinoma prostate.[52]

Akinloye et al (2009) observed that there was a positive correlation of serum total bilirubin, ALP and LPO levels with serum PSA concentration.[53]

Arsova-Sarafinovsk et al. demonstrated a similar pattern of change in markers of oxidative stress in patients from two different ethnicity. Serum MDA levels in carcinoma patients were found to be higher as compared to BPH patients and healthy controls.[11]

Hoque et al (2010) didn't observe any significant correlation between carcinoma prostate risk and levels of oxidized proteins.[54]

Battisit et al (2011) found TBARS levels to be high and CAT activity to be low in carcinoma patients than in controls.[55]

Barocas et al observed that F2-isoprosane levels were higher in carcinoma prostate and high-grade prostatic intraepithelial neoplasia patients (2011).[56]

Wozniak et al demonstrated lower GSH-Px activity in prostate cancer patients than in healthy controls (2012).[57]

2.3.2 Oxidative stress in Prostate Cancer[58]

Prostate cancer cells proliferating in the absence of androgens typically have an aggressive phenotype. Though multiple factors and signaling pathways have been implicated in the development of aggressive prostate cancer[59,60] the trigger for initiation of malignancy is still a topic of debate. Prostate cancer is mainly a disease of aging, therefore, progressive inherent or acquired changes in cellular metabolism occurring over the years may play a very important role in the development of this disease.

Hydroxyl radicals, peroxides and superoxide are ROS that are generated during everyday metabolic processes in a normal cell. ROS, generated either endogenously (mitochondria, metabolic process, inflammation etc.) or from external sources[9], play a vital role in regulating several biologic phenomena. While increased ROS generation has traditionally been associated with tissue injury or DNA damage which are general manifestations of pathological conditions associated with infection, aging, mitochondrial DNA mutations and cellular proliferation; new and exciting information points to an essential role for

increased ROS generation in several cellular processes associated with neoplastic transformation and aberrant growth and proliferation.[61,62]

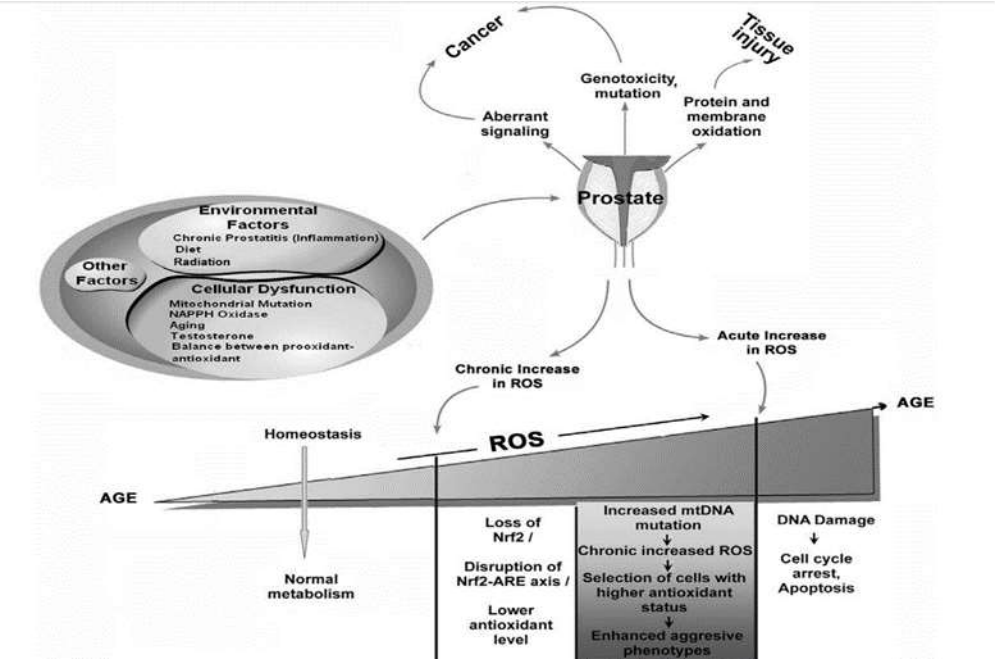

Image 2.6: Mechanisms of ROS production, and cellular response to ROS in prostate cells.[58]

Oxidative stress and one carbon metabolism are important contributary factors in etiopathogenesis in Prostate Neoplasm. Significant increase in oxidative stress and serum homocysteine level and no difference of serum vitamin B12 was found in BPH and prostate cancer cases compared to control group.[63]

The one-carbon metabolism network integrates several pathways that, together, play central roles in the biosynthesis of nucleic acids and lipids, amino acid and vitamin metabolism, the maintenance of redox status, methylation reactions and polyamine biogenesis. Because of the relevance of these pathways to cell growth and proliferation, they are critical not only for cellular homeostasis but also for tumorigenesis, and are therefore significant therapeutic targets.[21]

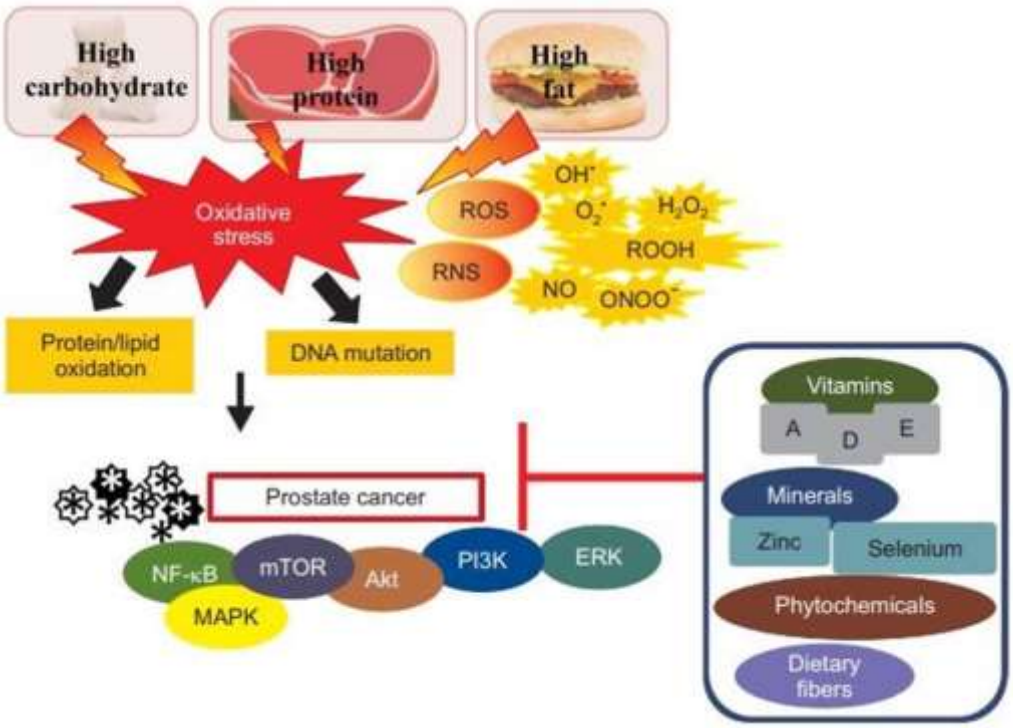

Image 2.7: The effect of oxidative stress on prostate cancer.

Androgen plays an important role in normal functioning, development, and differentiation of the prostate, and thus is considered to be the most powerful candidate that mediates reactive oxygen species (ROS) balance in the prostate.

The elevation of ROS has been associated with the progression and development of this disease. Conventional therapy has shown a high cure rate in patients with localized prostate cancer. Despite the patients respond favorably initially, this therapy fails to response in the advanced stage of the diseases even in the absence of androgens. Indeed, the onset and progression of prostate cancer could be prevented by changing dietary habits.[64]

Cancer initiation may be modulated by the nutrition-mediated elevation in ROS levels, which can stimulate cancer initiation by triggering DNA mutations, damage, and pro-oncogenic signaling. Therefore, the study, provided an overview of the relationship between nutrition, oxidative stress, and cancer initiation, and evaluated the impact of nutrient-mediated regulation of antioxidant capability against cancer therapy.[65]

Disturbed folate metabolism is associated with an increased risk of some cancers. Thus, Vitamin B12 and (in cohort studies) folate were associated with increased prostate cancer risk.[66]

2.3.3 One-Carbon Metabolism in Cancer

Cancer creates a demand and dependency on one-carbon metabolism. Proliferation of tumor cells not only requires increased DNA synthesis, but can also result in increased levels of reactive radical oxygen species (ROS), which are cytotoxic unless neutralized.[67,68]

Methyl group availability for methyltransferases that modulate gene expression via epigenetic mechanisms is influenced by flux within the folate cycle and methionine cycles.[4,6] In addition, synthesis of polyamines, which have been suggested to have oncogenic functions through regulating protein synthesis and proliferation[69,70] is SAM-dependent.

One-carbon metabolism involves a complex network with two central cycles: (1) the folate cycle; and (2) the methionine cycle. In the folate cycle, tetrahydrofolate (THF) acts as a carbon carrier donor for the synthesis of purines and thymidylates, which are vital for DNA synthesis and repair. The transfer of methyl groups from 5-methylTHF to homocysteine to form methionine links the two cycles. Methionine is then converted to S-adenosyl-methionine (SAM), the universal methyl donor for protein and DNA methyltransferase reactions. By donating a methyl group, SAM is converted to S-adenosyl-homocysteine (SAH), and subsequently to homocysteine to close the cycle.[21]

In addition to being recycled back to methionine, homocysteine can also be shunted to the trans sulphuration pathway where it is converted into cystathionine, a precursor of glutathione, an important cofactor in oxidation/reduction (redox) reactions that regulate the cellular redox state. SAM can also contribute to the synthesis of polyamines, which are small organic cations that regulate multiple biological processes, including, translation and proliferation, linking the methionine cycle with polyamine synthesis.[71,72]

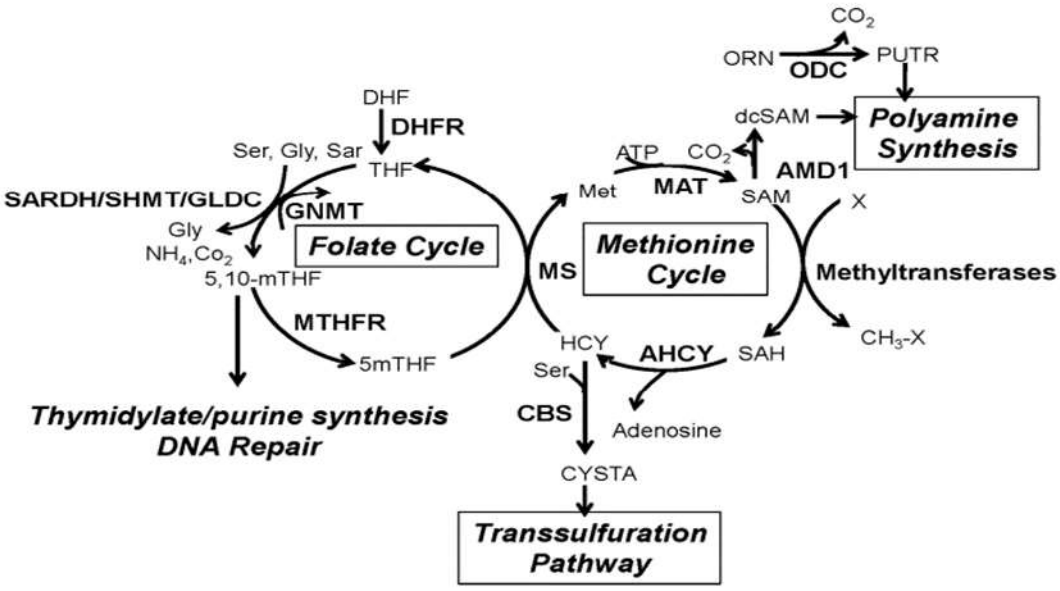

Image 2.8: One-carbon metabolism and associated pathways.

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CHAPTER: 4

MATERIAL & METHODS

4 MATERIALS AND METHODS

STUDY SETTING:

Department of Biochemistry, Santosh Medical College, Ghaziabad

ETHICAL CLEARANCE:

The necessary approval was obtained to conduct the study from the Santosh Medical College and Hospital, ethical committee, Ghaziabad. Patients were given an explanation regarding the intention of the study and informed written consent was obtained, confidentiality about their results was assured. Their participation was optional.

TYPE OF STUDY:

Hospital based study

STUDY DESIGN:

Cross Sectional study

DURATION OF STUDY:

The study was conducted for a duration of one year

SAMPLE SIZE:

Total 195 individuals were included in this study. The subjects were divided into three groups as follows:

Group 1 (Patients with BPH): Group 1 includes 65 patients with Benign Prostatic Hyperplasia (BPH) of the age group between 50 years – 90 years.

Group 2 (Patients with Prostate Neoplasm): Group 2 includes 65 patients with Prostate Neoplasm of the age group between 50 years – 90 years.

Group 3 (Normal Healthy Individuals): Group 3 includes 65 Normal Healthy Individuals of the age group between 50 years – 90 years.

Sample size was determined by using the formula:[73]

$$n = \frac{2\,(Z\ 1\text{-}\alpha/2 + Z\ 1\text{-}\beta)2\ \sigma 2}{(\mu 1 - \mu 2)2}$$

Where, $Z\alpha = 1.96$ at $\alpha = 5\%$ level of Significance

$Z\beta = 0.84$ at 80% Power of test

$\mu 1$ = Mean of Reference Group

$\mu 2$ = Mean of Test Group

σ = Standard Group

n = 65 each group

INCLUSION CRITERIA:

- ☑ Patients with Prostate Neoplasm of age group between 50-90 years.
- ☑ Controls: Normal healthy individuals of age group between 50-90 years.

EXCLUSION CRITERIA:

- ☒ Female Patients.
- ☒ Histologically confirmed cases of BHP or Prostate Cancer.
- ☒ Patients with other cancers.
- ☒ Patients with liver diseases.
- ☒ Patients on anti-oxidants or multi-vitamin medications
- ☒ Patients who have altered PSA level for any other prostrate pathology like prostatitis

STUDY LOCATION:

The present study was conducted in 195 subjects (both cases and controls). The subjects were selected from Department of Surgery, Santosh Medical College and study was performed in Department of Biochemistry, Santosh Medical College, Ghaziabad.

INFORMED CONSENT:

Before enrolling a subject for the study, all the study participants were given an explanation of the study and informed written consent was taken, either from the subject themselves or their attendants before enrolment into the study.

METHOD OF DATA COLLECTION:

- An informed consent was obtained from both cases and controls. (Annexure III)
- Demographic details and relevant information were collected by using structured proforma by interviewing both cases and controls. (Annexure II)
- Clinical evaluation was done for both cases and controls in the Hospital.
- Blood sample was collected from each case and control for relevant Biochemical investigations

METHOD:

SAMPLING:

- A 5 mL of venous blood sample was drawn from a large peripheral vein of each subject in EDTA vial following strict aseptic precautions.
- Serum was separated by centrifugation (3500 rpm for 5 minutes) and stored at -

20°c.

Image 4.1: Centrifuge

INVESTIGATIONS:

MDA	Thiobarbituric Acid (TBA) Method
Vitamin B12	Electrochemiluminescent Immune Assay (ECLIA) method

Homocysteine	Enzymatic method
AST	International Federation of Clinical Chemistry (IFCC) Method
ALT	International Federation of Clinical Chemistry (IFCC) Method
PSA	Electrochemiluminescent Immune Assay (ECLIA) method
Alkaline Phosphatase (ALP)	International Federation of Clinical Chemistry (IFCC) Method

Image 4.2: Cobas c 311 Chemistry analyzer

Image 4.3: Cobas e 411 Immunoassay analyzer

EXAMINATION OF BLOOD SAMPLE:

1. ESTIMATION OF MALONDIALDEHYDE (MDA) LEVEL IN SERUM

Methodology:

Plaser and Cushman (1996) Method

Principle:

Serum was deproteinized and the precipitate was treated with thio barbituric acid at 90ºC for 1 hour. The pink color formed gives the measure of thio barbituric acid reactive substance (TBARS) and Optical Density was read at 520 nm using Spectrophotometer.

2. ESTIMATION OF VITAMIN B12 LEVEL IN SERUM

Methodology:

Electrochemiluminescent Immune Assay (ECLIA) method

Principle:

1st incubation: By incubating the sample (9 µL) with the vitamin B12 pretreatment 1 and pretreatment 2, bound vitamin B12 is released

2nd incubation: By incubating the pretreated sample with the ruthenium-labeled intrinsic factor, a vitamin B12-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.

3rd incubation: After addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium-labeled intrinsic factor become occupied, with formation of a ruthenium-labeled intrinsic factor vitamin B12 biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a

photomultiplier

Results are determined via a calibration curve which is instruments pecifically generated by 2-point calibration and a master curve provided via the COBAS link.

3. ESTIMATION OF HOMOCYSTEINE LEVEL IN SERUM

Methodology:

Enzymatic method

Principle:

Test principle Homocysteine Enzymatic Assay is based on a novel enzyme cycling assay principle that assesses the co-substrate conversion product instead of assessing co-substrate or Hcy conversion products of Hcy. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), to form methionine (Met) and S-adenosylhomocysteine (SAH), catalyzed by a Hcy S-methyltransferase. SAH is assessed by coupled enzyme reactions where SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase, and Hcy is cycled into the Hcy conversion reaction to form a reaction cycle that amplifies the detection signal. The formed Ado is immediately hydrolyzed into inosine and ammonia. In the last step, the enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2-oxoglutarate and NADH to form NAD+. The concentration of Hcy in the

sample is directly proportional to the amount of NADH converted to NAD+ (ΔA340 nm).

4. ESTIMATION OF LIVER FUNCTION TEST (LFT)

A) SERUM ASPARTATE TRANSAMINASE (AST)

Methodology:

International Federation of Clinical Chemistry (IFCC) Method

Principle:

L-Aspartate + α-Oxoglutarate ⟶ Oxaloacetate + L- Glutamate

MDH Oxaloacetate + NADH + H+ ⟶ Malate + NAD+

B) SERUM ALANINE TRANSAMINASE (ALT)

Methodology:

International Federation of Clinical Chemistry (IFCC) Method

Principle:

L-Aspartate + α-Oxoglutarate ⟶ Pyruvate + L-Glutamate

LDH Pyruvate + NADH + H+ ⟶ L-Lactate + NAD+

C) SERUM ALKALINE PHOSPHATASE (ALP)

Methodology:

International Federation of Clinical Chemistry (IFCC) Method

Principle:

Colorimetric assay in accordance with a standardized method. In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

p-nitrophenyl phosphate + H_2O ⟶ phosphate + p-nitrophenol

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Image 4.4: Reagents for AST/ALT

Image 5: Reagents for Alkaline Phosphatase

5. SERUM PSA (PROSTATE SPECIFIC ANTIGEN)

Methodology:

Electrochemiluminescent Immune Assay (ECLIA) method

Sandwich principle: Total duration of assay: 18 minutes.

1st incubation: 20 µL of sample, a biotinylated monoclonal PSA-specific antibody, and a monoclonal PSA-specific antibody labeled with a ruthenium complex) react to form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. ▪ Results are determined via a calibration curve which is instrumentspecifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

STATISTICAL ANALYSIS:

The collected data were analyzed with IBM.SPSS statistics software 22.0 Version. To describe about the data descriptive statistics, frequency analysis, percentage analysis was used for categorical variables and the mean & S.D were used for continuous variables. To find the significant difference between the

bivariate samples in independent groups the unpaired sample t-test was used. To find the significance in categorical data, Chi-Square test was used. If the expected cell frequency is less than 5 in 2×2 tables, then the Fisher's Exact was used. In all the above statistical tools, the probability value of <0.05 is considered as significant level.

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CHAPTER: 5

RESULTS & OBSERVATIONS

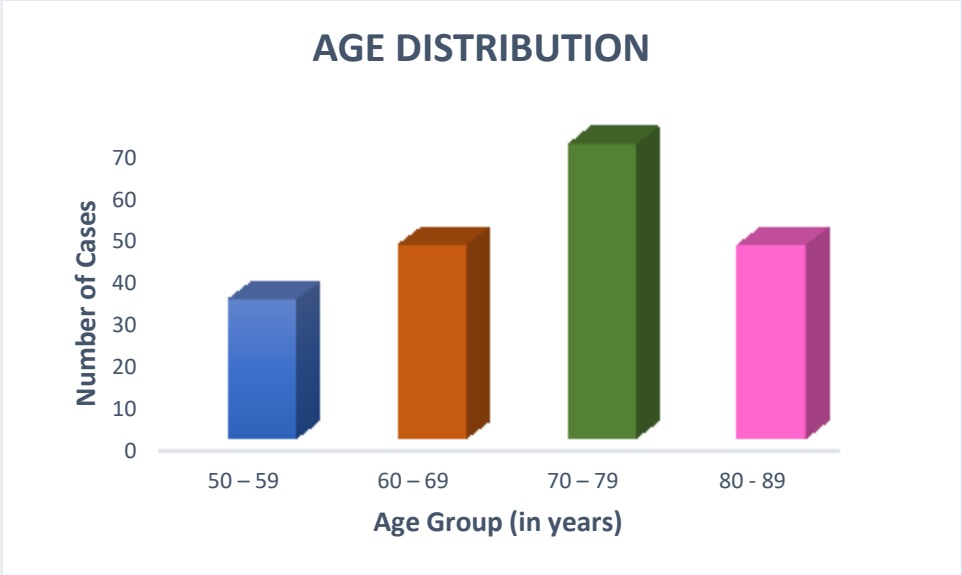
5 RESULTS AND OBSERVATIONS

The present study was conducted on 195 subjects which include patients with prostatic neoplasm, benign prostatic hyperplasia as well as normal healthy individuals after fulfilling inclusion and exclusion criteria and after calculating sample size. The relevant data were obtained after taking informed consent. The results and observations of the study are presented in tables and figures:

Table 5.1: Distribution of cases according to age

Age group (years)	Number of cases	Percentage
50 – 59	33	16.9
60 – 69	46	23.6
70 – 79	70	35.9
80 - 89	46	23.6
Total	**195**	**100**

Figure 5.1: Distribution of cases according to age



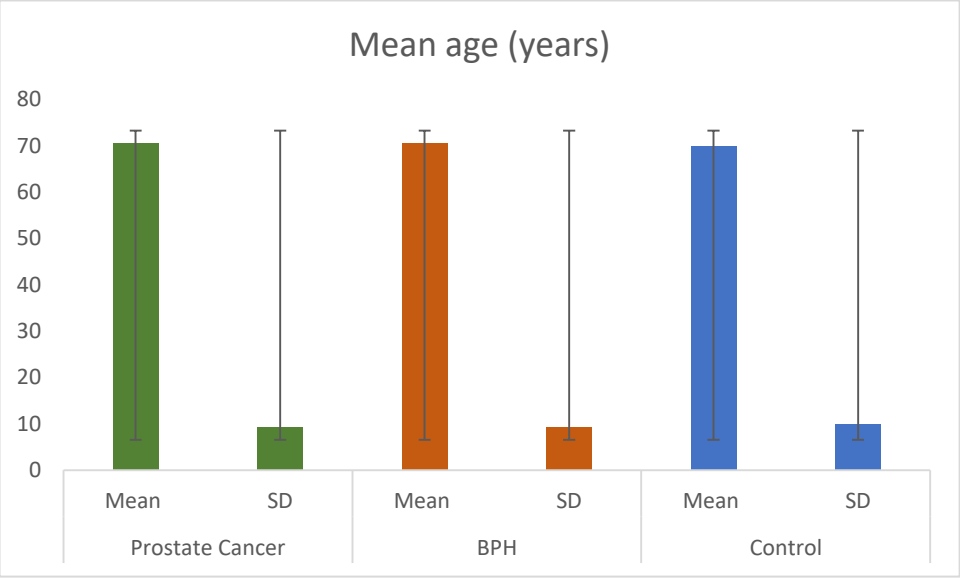
From Table 5.1 it was observed that maximum number of cases

(35.9%) were in the age group 70 – 79 years and minimum number of cases (16.9%) were in the age group 50 – 59 years.

Table 5.2: Mean age of Prostate cancer cases, controls and BPH

Condition	Age in years	
	Mean	**SD**
Prostate Cancer	70.55	9.32
BPH	70.57	9.19
Control	69.86	9.88

Figure 5.2: Mean age of cases and controls

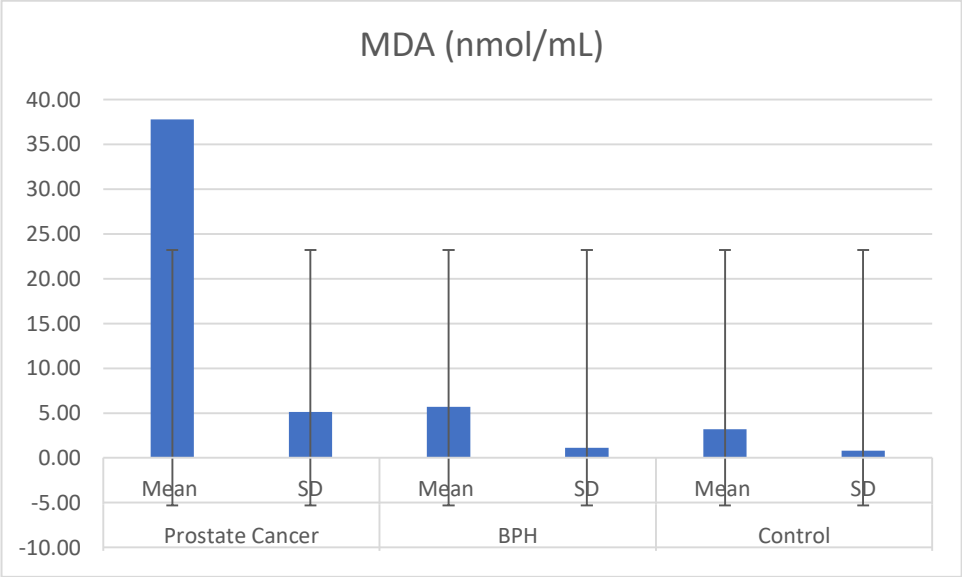


MDA level in cases and controls

Table 5.3: Mean ± SD of MDA (nmol/mL) in cases and controls

Condition	MDA levels (nmol/mL)		P Value
	Mean	SD	
Prostate Cancer	37.78	5.13	<0.05 (One way Anova)
BPH	5.69	1.13	
Control	3.19	0.81	

Figure 5.3: Mean ± SD of MDA (nmol/mL) in Cases and Controls



As represented by Table 5.3 and Figure 5.3, the Mean ± SD of MDA

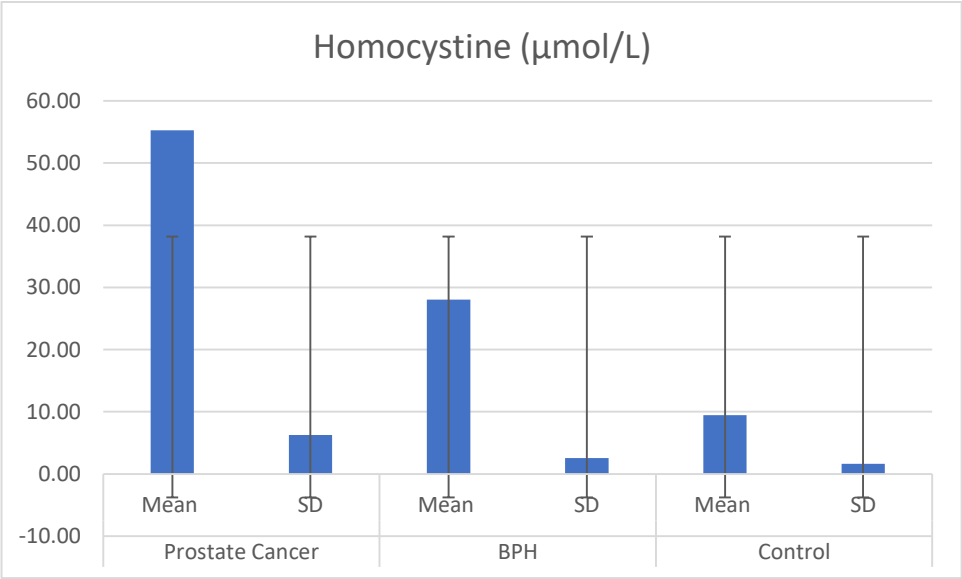
in Prostate cancer, BPH and healthy controls was found to be 37.78 ± 5.13, 5.69 ± 1.13 and 3.19 ± 0.81 respectively. The difference in MDA level in different groups was found to be statistically significant (p value < 0.05).

Homocysteine levels in cases and controls

Table 5.4: Mean ± SD of Homocysteine (µmol/L) in cases and controls

Condition	Homocystine levels (µmol/L)		P Value
	Mean	SD	
Prostate Cancer	55.26	6.27	<0.05 (One way Anova)
BPH	28.06	2.56	
Control	9.46	1.65	

Figure 5.4: Mean ± SD of Homocysteine (µmol/L) in cases and controls



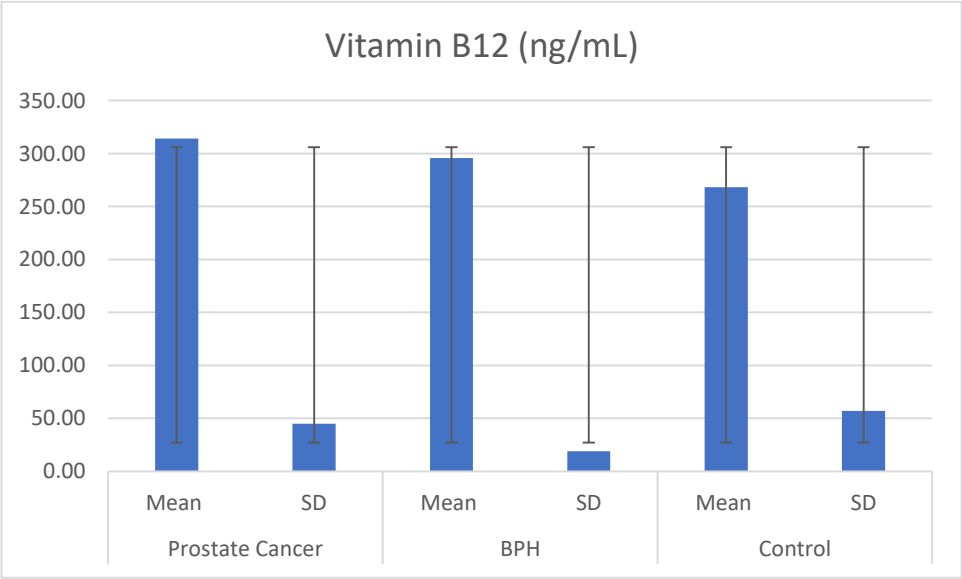
As represented by Table 5.4 and Figure 5.4, the Mean ± SD of Homocysteine in Prostate cancer, BPH and healthy controls was found to be 55.26 ± 6.27, 28.06 ± 2.56 and 9.46 ± 1.65 respectively. The difference in Homocysteine level in different groups was found to be statistically significant (p value < 0.05)

Vitamin B12 levels in cases and controls

Table 5.5: Mean ± SD of Vitamin B12 (ng/mL) in cases and controls

Condition	Vitamin B12 levels (ng/mL)		P Value
	Mean	SD	
Prostate Cancer	314.22	44.95	<0.05 (One way Anova)
BPH	295.58	18.96	
Control	268.00	57.10	

Figure 5.5: Mean ± SD of Vitamin B12 (ng/mL) in cases and controls



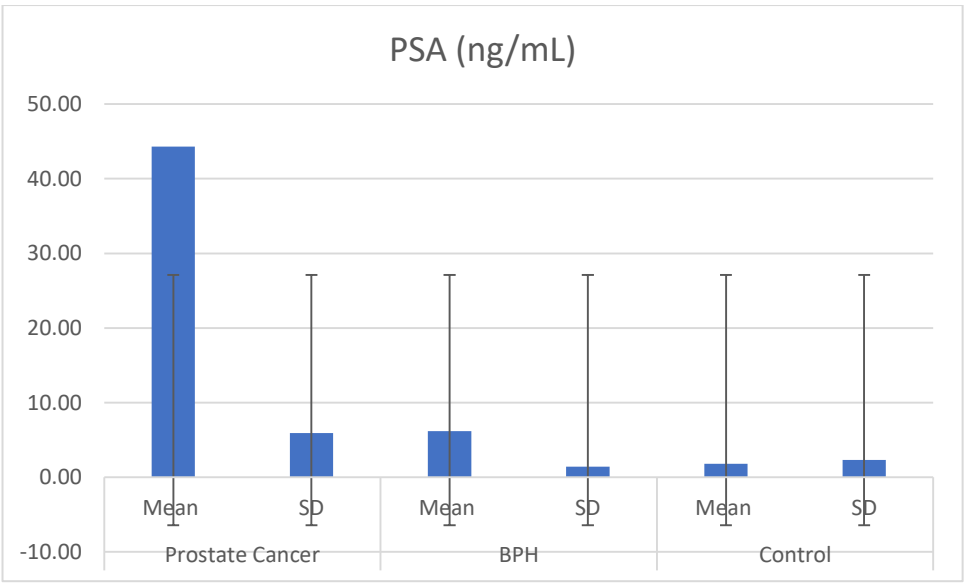
As represented by Table 5.5 and Figure 5.5, the Mean ± SD of Vitamin B12 in Prostate cancer, BPH and healthy controls was found to be 314.22 ± 44.95, 295.58 ± 18.96 and 268.0 ± 57.1 respectively. The difference in Vitamin B12 level in different groups was found to be statistically significant (p value < 0.05)

PSA levels in cases and controls

Table 5.6: Mean ± SD of PSA (ng/mL) in cases and controls

Condition	PSA levels (ng/mL)		P Value
	Mean	SD	
Prostate Cancer	44.32	5.92	<0.05 (One way Anova)
BPH	6.18	1.44	
Control	1.80	2.33	

Figure 5.6: Mean ± SD of PSA (ng/mL) in cases and controls



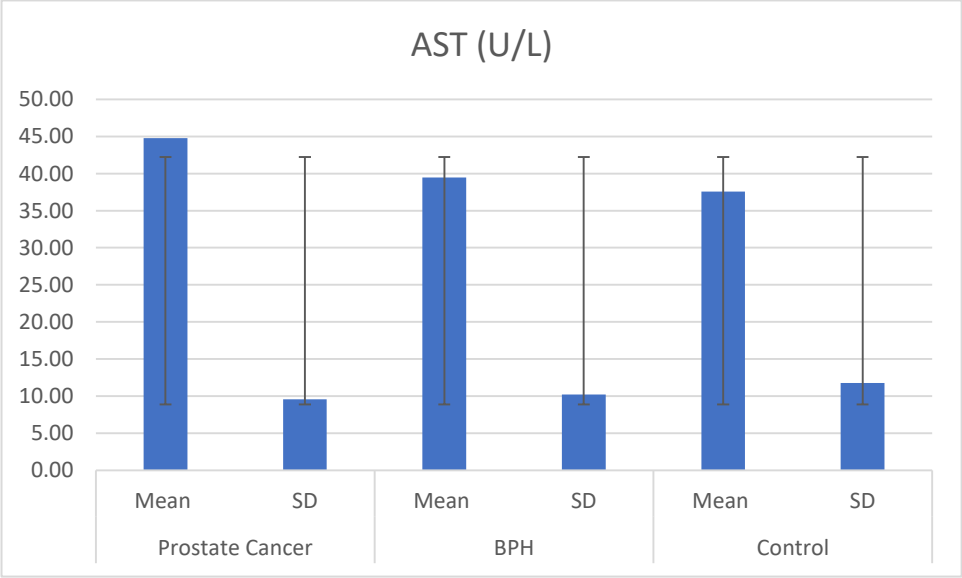
As represented by Table 5.6 and Figure 5.6, the Mean ± SD of PSA in Prostate cancer, BPH and healthy controls was found to be 44.32 ± 5.92, 6.18 ± 1.44 and 1.80 ± 2.33 respectively. The difference in PSA level in different groups was found to be statistically significant (p value < 0.05).

AST levels in cases and controls

Table 5.7: Mean ± SD of AST (U/L) in cases and controls

Condition	AST levels (U/L)		P Value
	Mean	SD	
Prostate Cancer	44.80	9.54	<0.05 (One way Anova)
BPH	39.49	10.23	
Control	37.57	11.75	

Figure 5.7: Mean ± SD of AST (U/L) in cases and controls



As represented by Table 5.7 and Figure 5.7, the Mean ± SD of AST in

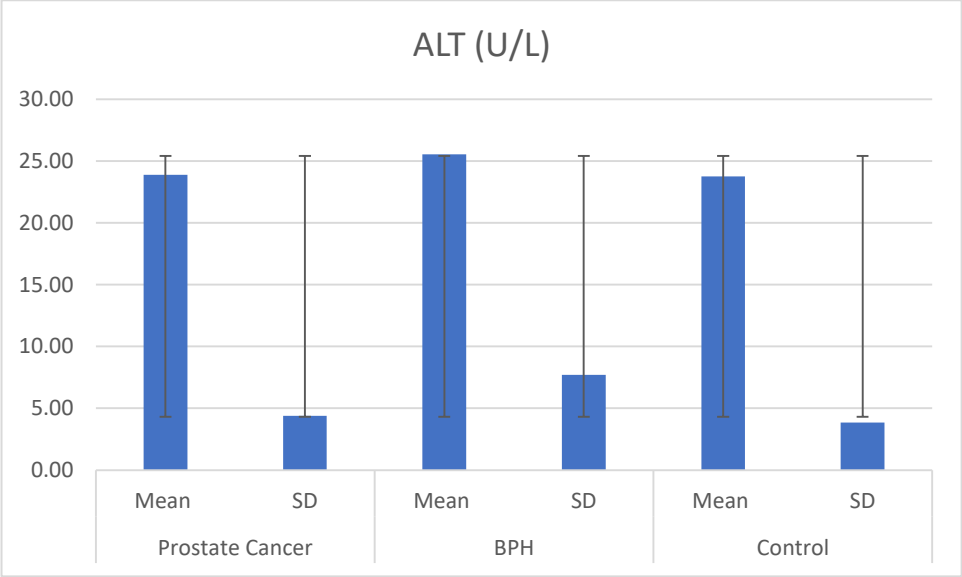
Prostate cancer, BPH and healthy controls was found to be 44.80 ± 9.54, 39.49 ± 10.23 and 37.57 ± 11.75 respectively. The difference in AST level in different groups was found to be statistically significant (p value < 0.05).

ALT levels in cases and controls

Table 5.8: Mean ± SD of ALT (U/L) in cases and controls

Condition	ALT levels (U/L)		P Value
	Mean	SD	
Prostate Cancer	23.89	4.39	>0.05 (One way Anova)
BPH	25.55	7.71	
Control	23.75	3.85	

Figure 5.8: Mean ± SD of ALT (U/L) in cases and controls



As represented by Table 5.8 and Figure 5.8, the Mean ± SD of ALT in

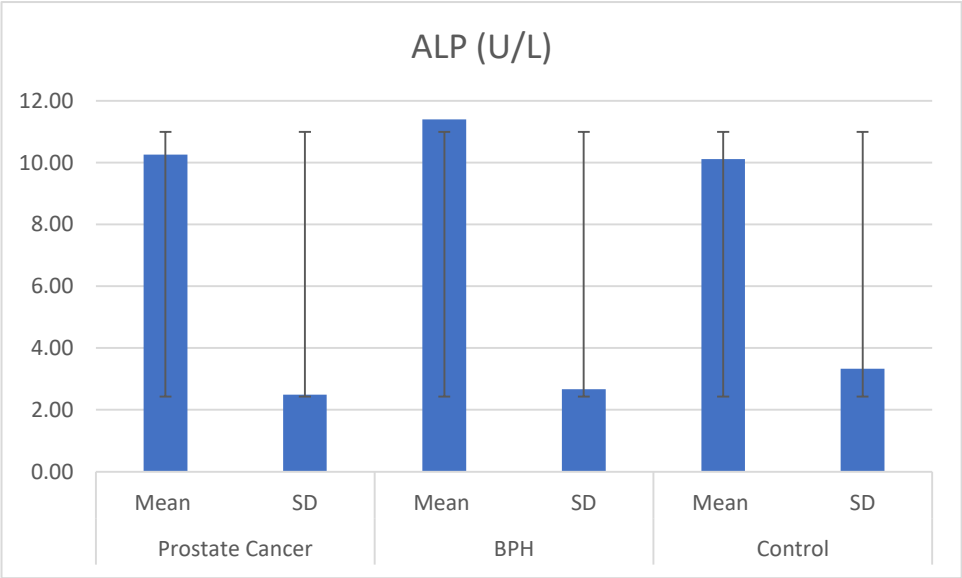
Prostate cancer, BPH and healthy controls was found to be 23.89 ± 4.39, 25.55 ± 7.71 and 23.75 ± 3.85 respectively. The difference in ALT level in different groups was not found to be statistically significant (p value > 0.05).

Alkaline Phosphatase levels in cases and controls

Table 5.9: Mean ± SD of ALP (U/L) in cases and controls

Condition	ALP levels (U/L)		P Value
	Mean	SD	
Prostate Cancer	10.26	2.49	<0.05 (One way Anova)
BPH	11.40	2.67	
Control	10.12	3.33	

Figure 5.9: Mean ± SD of ALP (U/L) in cases and controls



As represented by Table 5.8 and Figure 5.8, the Mean ± SD of ALP in Prostate cancer, BPH and healthy controls was found to be 10.26 ± 2.49, 11.40 ± 2.67 and 10.12 ± 3.33 respectively. The difference in ALP level in different groups was found to be statistically significant (p value < 0.05)

[redacted]***[redacted]

CHAPTER: 6

DISCUSSION

6 DISCUSSION

Age distribution

In our study, the maximum number of cases (35.9%) were in the age group 70 – 79 years and minimum number of cases (16.9%) were in the age group 50 – 59 years. In a study conducted by Bechis S et al.[74] 26% of men age ≥75 years presented with high-risk disease of Prostate cancer. With increasing age, men are significantly more likely to have high-risk prostate cancer.[1,75]

Mean age of Prostate cancer cases, controls and BPH

In our study, the mean age of Prostate Cancer, BPH and Control was 70.55 ± 9.32, 70.57 ± 9.19 and 69.86 ± 9.88 respectively. In a study conducted by Srivastava et al.[46] mean age of Prostate cancer patients were found to be 61.9 ± 11.4 and mean age of patients with BPH was 59.6 ± 8.4. In a study conducted by Sannigrahi S et al.[63] Mean age of control, BPH and Prostate cancer cases was 67.83±7.3, 69.55±7.6 and 66.00±9.4 years, respectively which is similar to our study. Thus, it is seen that older men are more likely to be diagnosed with high-risk Prostate Cancer and have lower overall survival. This risk increases significantly after the age of 50 in white men who have no family history of the disease and after the age of 40 in black men and men who have a close relative with Prostate Cancer.[76]

Serum MDA level

In our study, the Mean ± SD of MDA in Prostate cancer, BPH and healthy controls was found to be 37.78 ± 5.13, 5.69 ± 1.13 and 3.19 ± 0.81 respectively. The difference in MDA level in different groups was found to be statistically significant ($p<0.05$). In a study conducted by Arif M et al.[77] on BPH patients, mean value of MDA was 19.27 ± 4.84 µg/ml in BPH patients and normal control was 0.72 ± 0.02 µg/ml. The difference between BPH patients as compared to normal control was statistically significant ($p=0.002$). In a study conducted by Aryal M et al.[78] on Carcinoma Prostate Plasma MDA level showed 4.81±1.87 nmol/ml in BPH patients compared to 3.69±1.56 nmol/ml in healthy controls ($p < 0.001$). The MDA level, measured as a marker of oxidative stress was found significantly increased in BPH and prostate cancer patients when compared to control group.

Serum Homocysteine levels

In our study, the Mean ± SD of Homocysteine in Prostate cancer, BPH and healthy controls was found to be 55.26 ± 6.27, 28.06 ± 2.56 and 9.46 ± 1.65 respectively. The difference in Homocysteine levels in different groups was found to be statistically significant ($p<0.05$) In a study conducted by Sannigrahi S et al.[63] the levels of Homocysteine in BPH, Prostate cancer and controls was found to be 27.9 ± 4.86, 54.2 ± 11.9 and 9.51±3.03 respectively. In another study conducted by Collin SM et al.[66] they found that the median of Homocysteine in Prostate cancer patients was 11.1 (7.7-17.7). A study conducted by Hultdin J et al.[79] showed that Homocysteine levels in healthy Cases vs Prostate cancer patients

were 12.2 ± 3.1 vs. 13.2 ± 7.3 respectively.

Serum Vitamin B12 levels

In our study, the Mean ± SD of Vitamin B12 in Prostate cancer, BPH and healthy controls was found to be 314.22 ± 44.95, 295.58 ± 18.96 and 268.0 ± 57.1 respectively. The difference in Vitamin B12 level in different groups was found to be statistically significant ($p<0.05$). This is similar to a study conducted by Price AJ et al.[80] where they found that circulating concentrations of both folate and vitamin B12 were positively associated with risk of Prostate Cancer. In a similar study conducted by Sannigrahi S et al.[63] they found that the Mean ± SD of Vitamin B12 in Prostate cancer patients, BPH and healthy controls were 314.4 ± 68.7 94.7 ± 32.2 and 283.9 ± 90.4 respectively. The enzyme methionine synthase requires Vit B12 as coenzyme and the reaction also involves recycling of tetrahydrofolate which may be utilized for nucleotide biosynthesis. That is why, cancer patients are expected to have low plasma folate because tumor cells, due to high rate of cell division utilize folate from circulation to synthesize DNA (de-novo purine synthesis).[63]

Serum PSA levels

In our study, the Mean ± SD of PSA in Prostate cancer, BPH and healthy controls was found to be 44.32 ± 5.92, 6.18 ± 1.44 and 1.80 ± 2.33 respectively. The difference in PSA level in different groups was found to be statistically significant ($p<0.05$) In a study conducted by Malati T et al.[81] they found that among BPH group the mean PSA concentration was 3.6 ng/ml. Adenocarcinoma prostate patients prior to therapy revealed a mean PSA

concentration of 408 ng/ml. Their study clearly indicated rise of serum PSA with increasing burden of malignancy as almost all untreated patients and those with recurrence had very high PSA concentration. In another study done by William J et al.[82] they found that serum PSA measurement is a useful adjunct to rectal examination and ultrasonography in detecting prostate cancer. Although all three have the ability to predict cancer, the predictive value of serum PSA levels was greatest. Their results suggest that measurement of serum PSA and rectal examination combined, with the addition of ultrasonography in patients with abnormal findings, will provide a better method of detecting prostate cancer than rectal examination alone.

Serum AST/ALT levels

In our study, the Mean ± SD of AST in Prostate cancer, BPH and healthy controls was found to be 44.80 ± 9.54, 39.49 ± 10.23 and 37.57 ± 11.75 respectively. The difference in AST level in different groups was found to be statistically significant ($p<0.05$)

The Mean ± SD of ALT in Prostate cancer, BPH and healthy controls was found to be 23.89 ± 4.39, 25.55 ± 7.71 and 23.75 ± 3.85 respectively. The difference in ALT level in different groups was found to be statistically insignificant ($p>0.05$)

In a study conducted by Banez LL et al.[83] they found that LFTs were not associated with favorable pathological or biochemical outcomes in prostate cancer patients treated surgically. On the contrary, they found a modest association between SGOT levels and high-grade prostate cancer. Thus, at least in

radical prostatectomy patients, liver function does not seem to be of value in terms of predicting or affecting cancer-specific outcomes.

Serum Alkaline Phosphatase levels

In our study, the Mean ± SD of ALP in Prostate cancer, BPH and healthy controls was found to be 10.26 ± 2.49, 11.40 ± 2.67 and 10.12 ± 3.33 respectively. The difference in ALP level in different groups was found to be statistically significant ($p<0.05$)

In a similar study conducted by Ozu C et al.84 they found that the mean serum ALP levels were significantly elevated in patients with bone metastases suffering from prostate cancer ($p<0.05$) In a similar study conducted by Wei RJ et al.[85] on Prostate cancer, they found that serum levels of ALP increased in patients with bone metastases and combined detection of all markers could improve the positive predictive value.

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CHAPTER: 7

SUMMERY

7 SUMMARY

The present study "The association between Oxidative Stress and One Carbon Metabolism in Prostate Neoplasm in a tertiary care Hospital in North India" was conducted in 195 individuals (including patients with prostate cancer, benign prostatic hyperplasia as well as normal healthy individual in Department of Biochemistry, Santosh Medical College, Ghaziabad.

The aim of the study was to assess oxidative stress and one carbon metabolism in these cases to find out association with prostate neoplasm.

The data were collected after informed consent from the patients and relevant investigations were done. The results obtained were tabulated in appropriate tables and charts and appropriate statistical analysis was done to find out statistical significance.

Data was distributed as per age group (bracketed at 10 years interval).

- Maximum number of cases were found to be in the age group 70 – 79 years (36%).
- Minimum number of cases were found to be in the age group 50 – 59 years (17%)

- Mean age was found to be 70.55 ± 9.32 years in patients with Prostate cancer, 70.57 ± 9.19 years in patients with BPH and 69.68 ± 9.88 years in healthy controls respectively.

Serum MDA was estimated as a marker of oxidative stress.

Homocystine, and Vitamin B12 levels were estimated as indicator of one carbon metabolism.

Prostate specific antigen (PSA) was measured as novel biomarker of prostate health.

- Mean MDA level was found to be 37.78 ± 5.13, 5.69 ± 1.13 and 3.19 ± 0.81 in Prostate cancer, BPH and healthy controls respectively. The difference in MDA level in different groups was found to be statistically significant (p value < 0.05)

- Mean Homocystine level was found to be 55.26 ± 6.27, 28.06 ± 2.56 and 9.46 ± 1.65 in Prostate cancer, BPH and healthy controls respectively. The difference in different groups was found to be statistically significant (p value < 0.05)

- Mean Vitamin B12 level was found to be 314.22 ± 44.95, 295.58 ± 18.96 and 268.0 ± 57.1 in Prostate cancer, BPH and healthy controls respectively. The difference in Vitamin B12 level in different groups was found to be statistically significant (p value < 0.05)

- Mean PSA level was found to be 44.32 ± 5.92, 6.18 ± 1.44 and 1.80 ± 2.33 in Prostate cancer, BPH and healthy controls respectively. The difference in different groups was found to be statistically significant (p value < 0.05).

Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) were measured as a part of general investigation.

- Mean AST level was found to be 44.80 ± 9.54, 39.49 ± 10.23 and 37.57 ± 11.75 in Prostate cancer, BPH and healthy controls respectively. The difference in different groups was found to be statistically significant (p value < 0.05).

- Mean ALT level was found to be 23.89 ± 4.39, 25.55 ± 7.71 and 23.75 ± 3.85 in Prostate cancer, BPH and healthy controls respectively. However, this difference in ALT level in different groups was not found to be statistically significant (p value > 0.05).

- Mean ALP level was found to be 10.26 ± 2.49, 11.40 ± 2.67 and 10.12 ± 3.33in Prostate cancer, BPH and healthy controls respectively. The difference in different groups was found to be statistically significant (p value < 0.05).

[redacted]***[redacted]

CHAPTER: 8

CONCLUSION

8 CONCLUSION

From the present study "The association between Oxidative Stress and One Carbon Metabolism in Prostate Neoplasm in a tertiary care Hospital in North India" conducted in 195 patients and controls, it was observed that MDA levels were elevated in prostate cancer patients and BPH patients as compared to normal healthy individuals. This is suggestive of high level of oxidative stress associated with prostate cancer and BPH. Detailed exploration by extensive studies may help to find out possible role of antioxidants in modulating outcome of such patients.

In addition, it was observed that Homocystine and Vitamin B12 were high in patients with prostate cancer as well as BPH patients when compared with age matched healthy controls. This may be suggestive of possible role of once carbon metabolism with prostate cancer. Further longitudinal studies are required to establish a definite causal relationship.

The finding of present study, i.e. association of hyperhomocystinemia with carcinoma and benign hyperplasia of prostate needs to be validated by large prospective studies. This may help to establish the role of homocysteine as a novel marker for prostate neoplasm in addition to prostate specific antigen.

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ANNEXURE: i

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BIBLIOGRAPHY

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ANNEXURE: ..

PROFORMA

ii

PROFORMA

OPD/Clinic	Date:	Ref. By:	
Name:	Age:	Sex : Male	
Occupation:	Income:	Address:	
Educational Status			
	Y / N	Since	Quantity
Personal Habits Smoking Alcohol Tobacco Chewing Any other			
	Frequency of Intake per Day		
Dietary Habits Meat / Fruits / Vegetables High Fat Diet (Oil, Butter, Ghee) High Sugar Diet (Sweets, Sugar Intake) Vitamin C / Vitamin E Other			
Medication	Name of medicine dose since		
Presenting Complaints and History of Present Illness	Y / N	Duration	Remarks

Frequent Urination / Increased Urine			

Output (Polyuria)			
Excessive Thirst (Polydispia)			
Excessive Hunger (Polyphagia)			
Feeling of Tiredness / Fatigue / Cramps			
Weight Loss			
Fasting Symptoms (Stupor, Tremors, Nervousness, Sweating, Feeling Cold)			
Constipation / Diarrhea Blurred			
Vision / Cataract / Stye Conjuctival			
Infection / Iris Inflammation			
Corneal Ulceration / Glaucoma			
Recurrent Boils / Candidiasis			
Postural Unsteadiness / Drowsiness			
Distal Muscle Wasting / Weakness			
Sensory Loss / Pain / Tingling / Burning			
Corns / Ulcers / Skin Colour Changes			
Abdominal Pain / Nausea / Vomiting			
Burning Micturation			
Unhealthy Gums / Pus Discharge			

Coldness of Feet / Colour Changes Gastric Acidity / Fullness of Abdomen Delayed Wound Healing / UV Burns Sick Look / Dehydrated / Kussmaul Breathing			
Associated Diseases Obesity / Malnutrition (Emaciation) Hypertension Tuberculosis Acromegaly / Mumps Pregnancy / Big Baby Fever / Thyrotoxicosis Gall Stones / Colic			
Family History ***Diabetes Mellitus*** Tuberculosis Hypertension Obesity	Parent(s) / Sibling(s) / Second Degree Relative(s)		
	Name	Dose	Duration
Drug History Steroids Antihypertensive Drugs Diabetogenic Drugs Insulin			

Dietary Advise Breakfast Lunch Snacks Dinner				
				Remarks
Physical Activities At Work / Home At Leisure Time	Mild Walking	Moderate Sports	Severe Relaxation	
	Y / N			
Psychological History Stress Aggression Restlessness Quarrelling Temperamental Nature Hysteria				

[redacted]***[redacted]

ANNEXURE: iii

CONSENT FORM

iii CONSENT FORM

Name of the institution: Santosh Medical College, No.1 Santosh Nagar, Ghaziabad-201009, NCR Delhi

Telephone number of guide - 9990104307

Title of the study: "**The association between Oxidative Stress and One Carbon Metabolism in Prostate Neoplasm in a tertiary care Hospital in North India**"

Name of the participant:

……

Name of the principal investigator: Dr. Sweta Kumari

I, have been explained in my own language the aim and purpose of the study and the relevant investigation that would be carried out using my venous blood.

I have been given opportunity to express my doubts and all my doubts regarding the study have been cleared with proper explanation. I hereby give my consent to be a participant in the study.

Name of the participants:

Signature of the participants: **Date:**

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to clarify his/her doubts. I confirm that the individual has given consent freely.

Name of the witness: **Thumb impression of participant:**

Signature of the witness: **Date:**

ANNEXURE II(B)

सहमति पत्र

संस्थान का नाम : संतोष मैडिकल कालेज, नं0--1, संतोष नगर,गाजियाबाद--201009, एन.सी.आर. दिल्ली

मार्गदर्शक का संपर्क संख्या : 9990104907

शोध का विषय : उत्तर भारत में तृतीयक देखभाल अस्पताल में प्रोस्टेट नियोप्लाज्म में ऑक्सीडेटिव तनाव एवं एक कार्बन मैटाबोलिज्म के बीच संबंध का अध्ययन।

प्रतिभागी का नाम .....

मुख्य अन्वेक्षणकर्ता का नाम : डा0 श्वेता कुमारी

मैं ..... उम्र ..... वर्ष घोषणा करता / करती हूँ कि मुझे मेरी समझने वाली भाषा में इस शोध कार्य के सम्बन्ध में और मेरे शिरापरक रक्त पर होने वाले शोध के बारे में समझा दिया है।

मुझे मेरे संशय तथा संबंधित प्रश्नों के उत्तर मिल गए हैं तथा मैं इस शोध कार्य में हिस्सा लेने के लिए सहमत हूँ।

प्रतिभागी का नाम

प्रतिभागी के हस्ताक्षर **दिनांक**

<u>यदि अनपढ़ है तो</u>

मैं गवाही देता हूँ कि संभावित प्रतिभागी को मेरे सामने उनकी समझने वाली भाषा में उनके सभी संशयों व प्रश्नों के उत्तर दे दिए हैं तथा उक्त प्रतिभागी इस अध्ययन का हिस्सा बनने की सहमत है।

गवाह का नाम

गवाह के हस्ताक्षर **दिनांक**

[redacted]***[redacted]

ANNEXURE: ก

CASE RECORDS

SL NO	NAME	AGE (yrs)	MDA (nmol/mL)	VITAMIN B12 (ng/mL)	HOMOCYSTINE (µmol/L)	PSA (ng/mL)	AST (U/L)	ALT (U/L)	ALP (U/L)
1	AH	79	1.86	194.2	6.86	0.95	19	18	6
2	AK	58	2.36	256.3	7.69	0.96	46	19	6
3	LS	86	3.25	372.4	8.56	0.97	25	25	8
4	KJ	74	2.53	195.7	10.26	1.25	26	26	8
5	DB	70	2.59	196.5	11.56	1.28	35	26	11
6	RB	65	2.73	256.1	12.45	1.29	39	30	12
7	PK	62	4.53	286.8	7.59	2.01	41	18	18
8	SK	75	2.49	249.3	8.56	2	56	21	17
9	JU	80	3.52	362.4	9.58	2.03	20	25	16
10	QS	83	2.69	259.6	10.59	0.98	29	26	16
11	WS	75	4.23	248.3	11.56	0.93	30	18	6
12	TH	69	2.45	215	8.59	1.45	31	28	7
13	SC	67	3.25	210.3	9.45	1.28	35	30	7
14	DG	53	1.95	359.2	10.58	1.49	36	31	7
15	EF	64	2.65	348.1	11.56	1.58	37	24	17
16	BH	73	3.48	196.1	12.41	1.76	34	21	15
17	RT	88	4.52	200.5	9.65	2.01	45	20	13
18	JI	73	1.83	269.4	8.25	2.04	56	25	11
19	OP	63	2.56	261.2	7.45	1.58	49	18	10
20	SD	84	3.58	289.6	6.63	1.56	53	26	8
21	CV	82	4.23	345.4	8.69	1.49	21	30	6
22	WD	81	2.42	198.4	11.59	1.2	51	21	9
23	FH	56	3	196.4	10.85	1.67	45	24	6
24	RH	83	2.6	206.1	11.24	1.58	28	18	14
25	SG	73	3.41	215.7	8.69	1.86	21	24	12
26	BH	77	2.56	256.2	9.56	1.48	26	28	13
27	LM	70	2.5	269.2	10.45	1.59	34	31	14
28	LB	70	3.59	280.6	11.85	1.57	45	30	7
29	DS	60	4.12	284.5	6.89	1.28	57	26	6
30	EG	80	4	356.4	7.84	1.56	49	25	6
31	KL	59	3.86	263.5	23.51	3.59	19	18	7
32	LM	86	4.25	312.6	25.42	4.56	46	19	15
33	GH	76	5.26	314.6	31.52	4.59	25	25	15
34	TH	84	6.31	264.8	25.69	4.21	26	26	10
35	RG	75	7.21	263.2	26.34	5.85	39	26	10

36	LB	71	4.62	286.2	30.52	6.21	41	30	8
37	RC	74	5.32	267.2	25.62	7.45	56	18	9
38	PL	70	6.31	314.3	25.66	8.41	26	21	10
39	RK	59	4.78	310.2	26.69	7.52	35	30	7
40	LC	63	5.98	302.2	29.48	4.52	39	18	8
41	RS	62	5.54	268.9	30.44	5.26	41	21	9
42	PL	59	7.21	278.2	31.25	6.23	34	25	10
43	HT	65	3.64	289.4	30.22	7.45	45	26	10
44	BG	62	4.12	315.7	29.66	8.25	57	18	10
45	TR	60	5.78	289.3	26.89	4.25	49	30	11
46	LP	81	5.21	312.5	25.44	6.32	21	31	12
47	MH	82	6.32	289.4	26.89	5.25	51	24	16
48	RG	80	6.85	271.5	25.48	7.25	45	21	15
49	GH	57	6.95	296.3	31.56	8.12	28	20	14
50	TD	58	6.78	310.2	30.89	4.25	45	18	14
51	OP	68	27.56	256.3	43.21	33.56	19	18	8
52	SD	75	26.59	361.8	52.12	45.21	46	19	9
53	CV	80	42.36	296.4	61.56	51.26	25	25	15
54	RG	83	40.12	345.2	60.12	35.25	26	26	14
55	GH	75	41.15	346.5	49.63	41.26	35	26	6
56	TD	69	42.16	301.5	51.23	51.26	39	30	8
57	DB	73	43.12	263.1	63.12	33.67	38	30	10
58	RB	63	29.63	296.3	52.36	35.21	45	18	12
59	PK	84	42.3	314.2	48.63	41.25	46	21	14
60	SK	82	41.5	286.3	49.66	45.89	43	25	7
61	JU	81	40.2	259.5	50.12	51.26	35	26	6
62	LB	84	41.2	349.4	53.67	36.25	39	18	9
63	RC	75	36.2	369.4	61.56	45.21	41	19	10
64	PL	71	42.3	374.1	64.59	48.56	56	25	11
65	RK	74	32.6	259.4	65.23	47.56	20	26	12
66	PL	70	31.8	364.1	46.31	48.69	29	26	13
67	HT	59	37.5	348.6	51.26	41.2	49	30	14
68	CV	63	37.7	153.7	56.21	35.21	52	18	15
69	WD	60	36.4	286.3	65.12	36.27	56	30	5
70	FH	81	35.5	297.5	45.23	51.26	58	18	6

71	RH	82	29.6	361.5	56.23	48.56	43	21	7
72	IA	80	43.2	258.4	58.64	49.68	31	25	10
73	PG	57	41.2	362.1	61.26	48.65	31	26	11
74	DS	58	42.6	284.1	45.32	51.24	39	18	12
75	GKD	60	41.8	364.5	52.14	48.56	46	30	15
76	TB	80	40	289.4	56.21	51.26	36	31	14
77	LB	67	45.11	345.8	56.22	38.59	37	21	8
78	SB	63	38.56	301.5	49.63	45.23	34	24	9
79	SA	65	36.57	356.4	56.21	51.23	45	24	10
80	BKG	85	29.66	349.1	56.21	49.63	56	21	11
81	PG	59	34.54	312.4	48.56	41.25	49	20	12
82	SCD	85	41.21	322.5	56.21	35.21	57	25	15
83	SMR	82	42.64	344.8	62.12	51.23	38	31	8
84	SH	81	44.51	269.4	56.22	48.23	45	24	9
85	BB	75	41.28	341.8	63.21	49.63	46	21	10
86	VG	67	1.86	310.2	8.56	1.49	40	21	7
87	DGF	63	2.69	196.4	10.59	1.79	42	23	8
88	RDP	65	3.58	245.9	11.48	1.2	35	24	10
89	ER	85	4.16	256.4	12.48	1.23	29	26	11
90	EI	59	4.6	198.5	8.65	1.69	45	23	12
91	ASD	63	1.9	200.6	9.63	1.58	49	24	10
92	SD	76	2.56	258.6	10.7	1.59	52	21	11
93	PE	75	3.24	215.6	11.4	1.6	56	20	18
94	HL	70	3.14	245.7	7.84	1.11	58	19	15
95	LK	71	3.85	259.8	8.47	1.12	51	18	9
96	WE	66	2.69	267.3	9.78	0.96	19	31	8
97	IG	84	1.82	345.1	8.42	20.1	20	30	8
98	AG	79	4.2	358	8.21	2	31	21	11
99	RG	76	4.31	300	8.29	1.26	49	22	11
100	HT	58	2.59	314.5	8.74	1.36	36	25	11
101	JK	74	28.64	256.8	56.23	40.22	43	20	11
102	SS	70	30.45	346.1	48.56	38.54	39	18	8
103	MS	71	38.56	312.8	56.21	39.61	41	26	9
104	HM	76	42.64	311.5	58.22	40.21	56	30	10
105	PK	58	45.21	256.9	48.26	42.56	20	24	11

106	TG	65	28.63	286.3	59.54	45.63	29	21	12
107	LP	73	34.56	361.8	63.21	51.23	30	20	13
108	PU	58	39.63	378.2	60.21	36.23	45	25	14
109	TU	86	41.56	366.6	54.12	37.25	28	18	9
110	RS	81	42.6	255.8	54.11	48.63	45	21	10
111	DH	66	40.89	310.5	62.22	36.5	57	25	11
112	TD	60	35.26	256.3	58.55	51.26	38	26	8
113	SD	58	35.62	296.3	43.25	45.23	45	18	9
114	RT	53	36.45	299.7	57.21	48.56	46	30	8
115	LH	51	39.64	269.9	60.25	41.23	43	31	9
116	SD	56	3.65	312.5	25.69	5.21	57	26	12
117	RT	75	3.78	289.5	24.69	5.52	49	30	11
118	LH	86	3.89	294.5	25.5	5.55	21	21	12
119	PD	85	3.95	289.6	26.78	6.25	26	24	13
120	AS	82	4.12	294.5	27.55	7.25	34	24	14
121	AB	81	5.56	314.5	28.56	5.56	45	21	11
122	PK	75	5	302.3	29.63	5.69	28	20	10
123	PC	74	5.21	307.5	30.14	6.23	21	25	8
124	HM	70	6.21	310.5	31.2	7.45	26	18	7
125	TS	71	7.41	311.9	31.59	8.41	34	26	12
126	AD	85	7.15	289.6	26.56	8.25	40	30	11
127	TC	82	6.45	265.2	28.63	7.56	42	31	10
128	AC	81	5.75	256.3	29.64	7.12	35	24	14
129	PT	80	6.45	289.6	30.45	7.1	29	21	15
130	TG	59	6.45	315.8	31.52	7.15	36	20	11
131	GH	55	7.15	320.9	32.41	4.52	45	25	10
132	RD	56	6.35	324.5	31.56	4.25	46	20	9
133	DF	57	5.21	325.8	28.56	6.25	41	25	10
134	BH	62	4.45	289.6	26.56	4.26	46	18	7
135	CH	65	4.96	298.4	25.63	6.25	47	26	7
136	VG	65	3.75	356.2	7.49	1.46	48	25	10
137	TH	73	3.41	348.5	6.86	1.86	45	26	10
138	LO	58	4.13	359.4	7.21	1.95	35	24	11
139	PK	86	1.86	237.5	8.52	1.36	29	27	7
140	FG	81	3.15	196.8	9.61	1.26	19	28	8

141	RT	54	3.21	274.5	10.41	1.49	54	29	9
142	KL	74	3.56	296.9	10.2	1.56	19	25	6
143	HL	56	4.21	359.2	10	1.86	25	21	7
144	TH	64	4.2	345.1	11.21	1.96	54	20	14
145	BH	66	2.69	345	12.42	2.01	19	18	12
146	JK	60	3.15	278.6	10.49	2.03	26	19	8
147	KL	58	3.48	214.2	9.54	1.67	28	20	8
148	FB	53	3.78	196.4	8.76	1.26	31	21	12
149	KO	51	4.13	215.7	7.21	1.59	34	22	13
150	FB	53	4.56	220	6.81	1.69	45	22	10
151	TH	65	4	225.2	7.45	1.56	46	21	9
152	RH	63	3.27	256.3	8.45	1.85	48	20	8
153	SD	75	1.96	248.4	9.41	1.97	26	25	7
154	FH	82	2.67	289.6	10.56	0.96	38	26	6
155	KP	74	3.42	348.1	11.47	0.98	42	25	11
156	JD	66	4.58	276.4	24.56	7.15	51	30	10
157	AS	62	4.12	296.3	26.66	7.15	46	21	16
158	SH	63	5.89	297.5	27.45	6.23	28	24	11
159	RT	64	6.12	315.4	31.25	4.15	28	18	16
160	DF	72	7.12	318.4	30.56	3.89	51	25	14
161	DC	71	4.52	321.7	29.63	4.12	53	18	14
162	BG	70	5.26	267.4	28.52	6.32	51	26	10
163	SH	76	6.15	296.3	24.12	7.25	45	30	10
164	DS	74	7.13	314.5	23.09	8.12	28	21	12
165	GD	71	7.56	267.1	23.56	8.2	45	26	14
166	TB	70	7.45	289.1	25.12	8.23	57	25	14
167	PG	59	6.21	298.4	26.75	4.21	38	21	13
168	IA	56	5.12	288.6	31.45	5.26	45	23	15
169	SB	85	4.89	299.4	30.4	6.23	46	24	14
170	LK	88	5.23	300.8	30.9	7.22	43	26	10

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171	PD	53	42.5	310.2	64.11	40.85	36	30	10
172	HL	65	41.29	311.5	58.74	43.23	45	31	11
173	TH	63	35.25	348.5	46.21	51.23	46	24	12
174	BH	63	44.14	259.6	52.61	48.63	41	21	15
175	JK	64	28.69	265.3	62.55	38.56	46	20	8
176	KL	72	30.12	298.5	46.22	48.63	47	18	9
177	SD	71	31.56	300.5	63.25	49.63	51	26	10
178	FH	70	38.56	321.6	60.11	51.29	29	25	11
179	KP	76	35.6	359.4	61.1	48.62	30	18	10
180	CH	74	42.66	378.6	43.21	38.55	31	26	11
181	JD	75	41.25	381.8	45.29	51.71	35	30	8
182	AS	70	40.12	359.6	48.52	37.45	36	21	9
183	SH	71	28.63	389.9	57.25	34.12	47	18	8
184	MJH	66	35.18	290.9	57.15	47.51	51	26	8
185	KB	84	39.47	289.6	60.15	44.22	46	30	10
186	JH	74	6.89	300.1	26.56	6.66	31	23	7
187	GT	70	7.15	324.8	29.6	7.56	31	24	7
188	BG	71	4.45	322.2	30.45	6.66	39	21	11
189	PG	72	5.12	310.2	29.64	7.15	46	20	12
190	SA	73	6.87	298.6	26.45	8.21	56	39	14
191	SC	71	6.21	263.4	25.26	4.25	48	46	15
192	AD	72	6.1	286.9	26.31	3.78	40	56	11
193	RK	70	7.15	267.5	26.96	6.45	46	48	10
194	AD	73	6.15	298.8	25.64	7.52	31	40	16
195	JD	74	4.89	269.9	31.25	4.25	28	46	11

ANNEXURE: ד ע

ABBREVIATIONS

v ABBREVIATIONS

Serial No.	Abbreviation	Full form
1	BPH	Benign Prostatic Hyperplasia
2	ROS	Reactive Oxygen Species
3	MDA	Malondialdehyde
4	Hcy	Homocysteine
5	PSA	Prostate Specific Antigen
6	PIN	Prostatic Intraepithelial Neoplasia
7	PGF	Prostate Growth Factor
8	SOD	Superoxide dismutase
9	TBRAS	Thiobarbituric Acid Reactive Species
10	SAM	S-adenosyl methionine
11	SAH	S-adenosyl homocysteine
12	THF	Tetrahydrofolate
13	TBA	Thiobarbituric acid
14	IFCC	International Federation of Clinical Chemistry
15	ECLIA	Electrochemiluminescence Immunoassay

16	AST	Aspartate transaminase
17	ALT	Alanine transaminase
18	ALP	Alkaline phosphatase

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